Respiratory Medicine (2009) **103**, 601–606

available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/rmed

Relation of sputum colour to bacterial load in acute exacerbations of COPD☆

M.G.J. Brusse-Keizer [a,*], A.J. Grotenhuis [a], H.A.M. Kerstjens [b,c], M.C. Telgen [a], J. van der Palen [a], M.G.R. Hendrix [d], P.D.L.P.M. van der Valk [a]

[a] *Department of Pulmonary Medicine, Medisch Spectrum Twente, P.O Box 50000, 7500 KA, Enschede, the Netherlands*
[b] *Department of Pulmonary Medicine, University Medical Center Groningen, P.O Box 30.001, 9700 RB Groningen, the Netherlands*
[c] *University of Groningen, P.O Box 72, 9700 AB Groningen, the Netherlands*
[d] *Regional Laboratory of Public Health, P.O Box 377, 7500 AJ Enschede, the Netherlands*

Received 8 July 2008; accepted 14 October 2008
Available online 22 November 2008

KEYWORDS
COPD;
Sputum colour;
Bacterial load;
Antibiotics

Summary
Background: When COPD patients present with an exacerbation, one cannot verify a bacterial cause of an exacerbation without time-consuming laboratory analyses. This makes it difficult to decide up front if antibiotic treatment is needed. Therefore, in clinical practice sputum colour and purulence are often used.
Objective: To determine whether sputum colour and purulence, assessed by the Stockley colour chart, correlated with overall bacterial load in COPD patients admitted for an exacerbation. To check the robustness of the colour and purulence assessment, we correlated the changes in these parameters and the corresponding change in bacterial load in sputum over the first seven days of hospitalisation.
Methods: Twenty-two COPD patients admitted to the hospital for an exacerbation were included. During the first seven days daily sputum samples were collected.
Results: A very weak association between bacterial load and sputum colour was found. There was no difference in bacterial load between patients with purulent sputum or not. Also, no consistent relationship between change in sputum colour and change in bacterial load during admission was found.

☆ Originality and clinical relevance of our paper: In clinical practice sputum colour or purulence is often used to decide if antibiotic treatment in COPD exacerbations is needed. This study, however, shows that there is only a very weak association between sputum colour and bacterial load in sputum of COPD patients. The distinction between purulent and mucoid sputum at exacerbation is insufficient for distinction between patients who are likely to benefit from antibiotic therapy and those who are not.

* Corresponding author. Tel.: +31 53 4872643; fax: +31 53 4872676.
E-mail address: mbrussekeizer@euronet.nl (M.G.J. Brusse-Keizer).

0954-6111/$ - see front matter
doi:10.1016/j.rmed.2008.10.012

Conclusions: The very weak association between bacterial load and sputum colour confirms concerns over the usefulness of the colour chart. The distinction between purulent and mucoid sputum at exacerbation is insufficient for distinction between patients who are likely to benefit from antibiotic therapy and those who are not. Complementary studies are needed to determine which other, easily measurable factors can be used as predictors for an indication for use of antibiotics; sputum colour is not the one.

Introduction

Morbidity and mortality among patients with chronic obstructive pulmonary disease (COPD) are for an important part related to acute exacerbations, which occur on average one to three times a year but much more frequently in some patients.[1,2] Exacerbations are characterised by a heterogeneous aetiology. Recent studies showed that at least one-third of the exacerbations might be triggered by viral infections.[3–6] Furthermore, bacteria play an important role in the onset of exacerbations.[7] Several studies have shown the presence of potential pathogenic micro-organisms (PPMs) in approximately 50% of exacerbations.[8,9] On the other hand, these PPMs might also colonise the airways of COPD patients.

Treatment of exacerbations with antibiotics is usually empirical. At clinical presentation it is hard to predict and verify a bacterial cause without time-consuming laboratory analyses, which makes it difficult to decide up front if antibiotic treatment is needed.[10] According to the GOLD criteria, patients with an exacerbation who meet the Anthonisen type I criteria (increase in dyspnoea, sputum volume and sputum purulence) benefit from antibiotic treatment.[11] However, Van der Valk et al. reported that an Anthonisen type I exacerbation does not predict a bacterial origin of an exacerbation and that sputum purulence was not associated with a bacterial infection.[1]

Since purulence is subjective and not clearly defined, the use of colour charts has been suggested. Stockley et al. designed and validated a sputum colour chart by which a distinction between purulent and mucoid exacerbations can be made.[12] Also Allegra et al. provided additional evidence that purulent sputum is associated with bacterial growth in the airways of patients with moderate to severe COPD. They, however, recorded that also in mucoid sputum presence of bacterial growth was very common (78%).[13] Results of a study by Soler et al. showed that self-reported purulence in the sputum predicts the presence of bacteria at concentrations in the airways of $\geq 10^2$ CFU/mL for protected specimen brush specimens.[14]

So, contradictory data and opinions about the association between sputum purulence and bacterial involvement in exacerbations result in uncertainty whether to use sputum purulence as an indicator for antibiotic treatment. The aim of the present study was to determine whether sputum colour and purulence, as assessed by the nine-point Stockley colour chart, correlate with bacterial load in patients admitted for an exacerbation of COPD. To check the robustness of the colour and purulence assessment, the changes in these parameters and the corresponding change in bacterial load in sputum over the first seven days of hospitalisation were correlated.

Methods

Patients and study design

Patients hospitalised from April 2007 through June 2007 for an exacerbation of COPD at the in-patient pulmonary department of Medisch Spectrum Twente in Enschede, the Netherlands were recruited. This study is part of our COPD cohort study, the COMIC study. Recruitment criteria were: 1) a clinical diagnosis of COPD as defined by the GOLD criteria, 2) admitted with signs and symptoms of an exacerbation of COPD, defined as an acute negative change from the baseline, reported by the patient, in dyspnoea and/or sputum volume and/or colour of sputum (yellowish or greenish sputum) and/or cough, which warranted additional treatment of prednisolone with or without antibiotics by a physician 3) current or former smoker, 4) age 40 years or over, 5) no pneumonia, defined as an acute respiratory tract illness associated with radiographic shadowing on a chest radiograph which was neither pre-existing nor of any other cause, 6) no maintenance treatment with antibiotics, 7) able to produce spontaneous sputum, and 8) no other medical condition with low survival or a serious psychiatric morbidity.

All patients included received care according to the hospital protocol for treatment of COPD exacerbations. The hospital's medical ethical committee approved the study. All patients provided written informed consent.

Baseline characteristics

Demographic and baseline data were collected using medical records. Smoking history was determined by the Vlagtwedde Questionnaire.[15] Exacerbation frequency in the preceding year and courses of antibiotics and prednisolone during the last four weeks before admission were registered from pharmacy records. Exacerbation frequency was estimated by counting the number of separate courses of antibiotics or prednisolone patients used for lung associated illness. Data on antibiotics and prednisolone during the first seven hospital days were obtained from hospital pharmacy records.

Sputum colour and microbiology

During the first seven days of admission, spontaneously expectorated sputum samples were collected. Samples

were collected once a day, preferably in the morning. Immediately after collection, sputum colour was determined according to the Stockley protocol (BronkoTest, Heredilab Inc., Salt Lake City, UT, USA).[12]

A Gram's stain and quantitative culture were performed for all collected sputum samples. Additionally, a semi-quantitative culture was performed for each patient's first sputum sample after admission. Bacterial load was defined as total amount of CFU/mL. Infection was defined by the presence of PPM in pure culture or as the presence of one or more PPM in excess (one log or more) to normal microbiological flora in sputum. Bacterial colonisation was defined as the presence of PPM in culture in equal amount or less compared to normal microbiologic flora in sputum.[1,16]

One day after admission a 10 cc blood sample was obtained. In all sputum and blood samples IL-6, IL-8 and IL-10 were quantified using PeliKine Compact™ human sandwich ELISA kits (Sanquin, CLB, Amsterdam, the Netherlands). C-reactive protein (CRP) level in sputum and blood was determined using the NycoCard® CRP Single Test (Clindia Diagnostics©). MPO enzymatic activity in sputum was determined by colorimetric change in absorbance during a reaction with O-Dianisidine dihydrochloride (Sigma–Aldrich©).

Statistical analysis

Baseline characteristics were reported as means ± SD for continuous variables or as numbers with percentages for categorical variables. Not normally distributed variables were reported as median with range. For non-parametric variables we applied a natural logarithm to obtain a normal distribution. If this was ineffective, variables were dichotomised based on median values.

Linear regression analysis was used to test the relation between sputum colour and bacterial load. Correlation coefficients were calculated to identify variables associated with sputum colour. In case of dichotomous variables this was performed by *t*-tests. The a priori list of potential confounding variables included: IL-6, IL-10 and CRP in sputum and blood, result of semi-quantitative culture of the first sputum sample (no colonisation and no infection versus colonisation or infection), smoking status, use of antibiotics or prednisolone during the four weeks prior to admission (yes/no) and use of antibiotics during the first seven days of admission (yes/no). Variables associated with sputum colour with $p < 0.15$ were subsequently tested for an association with bacterial load and considered as potential confounders when $p < 0.15$. Also of the variables IL-8, MPO in sputum, number of leukocytes in sputum, we analysed the association with both sputum colour and bacterial load. Since these variables are in the assumed pathway they were not entered in the multivariate model. We started the multivariate model with all potential confounders. Variables with the highest *p*-value were eliminated step by step, and at each step we verified whether the beta-coefficient of sputum colour had not changed by 10% or more from its initial value. The interaction between sputum colour and antibiotic use during the four weeks prior to admission and interaction between sputum colour and use of antibiotics during the first seven days of admission were assessed by adding these interaction variables into the multivariate model.

In a secondary analysis the association between sputum purulence and bacterial load was tested. Sputum with a colour value ≤2 was defined as mucoid and sputum colour ≥3 was defined as purulent. Potential confounding variables were identified by an essentially similar approach as described above, but the formal testing of associations was now performed with *t*-tests or Mann–Whitney *U*-tests as appropriate for continuous variables and by Chi-square tests or Fisher's Exact tests as appropriate for between-group comparisons of categorical variables. The association of variables in the pathway with sputum purulence and bacterial load were analysed. Interaction between sputum purulence and antibiotic use during the four weeks prior to admission and interaction between sputum purulence and use of antibiotics during the first seven days of admission were assessed by adding these interaction variables into the multivariate model.

Repeated measurements analysis was performed to test for change in sputum colour and bacterial load during the first seven days of admission. Finally, we looked at the relationship between a change in sputum colour and a change in bacterial load during hospital admission by means of scatter plots and correlation coefficients.

Data-analysis was performed with the statistical package SPSS/PC for Windows (version 12.0.1) (SPSS, Inc., Chicago, IL).

Results

Table 1 shows the baseline characteristics of the 22 included patients. In total, 124 sputum samples were obtained.

Relationship between sputum colour and bacterial load

The univariate linear regression analysis showed no significant relation between sputum colour and bacterial load ($p = 0.16$). Variables univariately associated with sputum colour were: IL-8 in sputum, MPO in sputum, leucocytes in sputum (yes/no), CRP in blood (≤14 versus >14 mg/L), use of antibiotics during the first seven days of admission and result of semi-quantitative culture of the first sputum sample. Of these variables, IL-8 in sputum, MPO in sputum and use of antibiotics during the first seven days of admission were also associated with bacterial load. The association between bacterial load and sputum colour was only adjusted for the use of antibiotics, since IL-8 and MPO are part of the assumed pathway between bacterial load and sputum colour, and adjustment for these factors would make a potential relationship disappear. There was no interaction between sputum colour and antibiotic use during the four weeks prior to admission, nor was there an interaction between sputum colour and use of antibiotics during the first seven days of admission. The multivariate model showed a significant positive regression coefficient for sputum colour (0.41 (95% CI: 0.01–0.81; $p = 0.045$)) and a significant negative coefficient for antibiotic use during admission (−2.19 (95% CI: −3.67 to −0.71; $p = 0.004$)). Both regression coefficients are on a log base. The explained variance of this model was only 0.087.

Table 1 Baseline characteristics of participating COPD patients.

	$N = 22$
Mean age at enrolment in years (SD)	68 (10)
Gender (Number (%))	
Male	10 (45)
Female	12 (55)
Smoking status (Number (%))	
Current smokers	12 (55)
Ex-smokers	10 (45)
Median exacerbation frequency/year (range)[a]	1.5 (0—5)
GOLD classification (Number (%))	
GOLD stage 1	1 (5)
GOLD stage 2	5 (23)
GOLD stage 3	10 (45)
GOLD stage 4	6 (27)
Lung function (post-bronchodilator)	
Median FEV_1 in litres (range)	0.95 (0.43—2.97)
Median FEV_1 as % of predicted (range)	36 (19—83)
Mean FEV_1/VC in % (SD)	40 (13)
Previous treatment (Number (%))	
Antibiotics <4 weeks before study entry	15 (68)
Finished course of antibiotics before admission	10 (45)
Still receiving antibiotics at admission	5 (23)
Prednisolone <4 weeks before study entry	14 (64)
Finished course of prednisolone before admission	11 (50)
Still receiving prednisolone at admission	3 (14)
Prednisolone maintenance therapy	2 (9.1)

FEV_1 = Forced Expiratory Volume in 1 s; IVC = Inspiratory Vital Capacity; GOLD = Global Initiative on Obstructive Lung Disease.

[a] Exacerbation frequency refers to the number of exacerbations during the year preceding admission and is based on the number of separate courses of antibiotics or prednisolone prescribed for lung associated illness according to the database of the patients' pharmacy.

Relationship between sputum purulence and bacterial load

The univariate linear regression analysis showed no significant relationship between sputum purulence and bacterial load ($p = 0.64$). Variables univariately associated with sputum purulence were: IL-8 in sputum, MPO in sputum and leucocytes in sputum (yes/no). IL-8 in sputum and MPO in sputum were also associated with bacterial load. Since, IL-8 and MPO are both part of the assumed pathway between bacterial load and sputum purulence, these variables were again not included in the multivariate analysis and no potential confounders remained. Furthermore there was no interaction between sputum purulence and antibiotic use during the four weeks prior to admission, nor was there interaction between sputum purulence and use of antibiotics during the first seven days of admission.

Longitudinal change in sputum colour and bacterial load during admission

Repeated measurements analysis showed no change in sputum colour ($p = 0.16$) and bacterial load ($p = 0.99$) during the first seven days of admission. Fig. 1 shows the mean value of sputum colour and bacterial load at days 2, 4 and 6 of admission. Fig. 2 a and b shows respectively the change in colour and the change in bacterial load during the first seven days of admission on a patient level.

Relationship between change in sputum colour and change in bacterial load

There was no consistent relationship between change in sputum colour and change in bacterial load during admission, as judged by the constructed scatter plots and correlation coefficients comparing baseline data with follow-up days and between consecutive follow-up days. The correlation coefficients varied from −0.819 to 0.884.

Discussion

This study found a significant but very weak association between bacterial load and sputum colour after correction for confounding by antibiotic use during admission. Only 8.7% of the variance in bacterial load could be explained by this model. Furthermore when we dichotomised the nine-point score of sputum colour into purulent and mucoid sputum, the relationship with bacterial load vanished completely. Also, no consistent relationship between change in sputum colour and change in bacterial load during admission was found.

Our findings on the relationship between sputum colour and purulence with bacterial load are in contrast to the results of other study groups. Stockley et al. showed that the presence of purulent sputum was 94.4% sensitive and 77.0% specific for the yield of a high bacterial load. Soler et al., who looked at the self-reported presence of purulent sputum, concluded that the self-reported presence of purulent sputum predicted the presence of bacterial infection in the distal airways.[14] Allegra et al. found an association between purulent sputum and bacterial growth, in which growth was classified as the presence of $>10^6$ CFU/mL.[13] They, however, did not only found a high number of positive cultures in purulent sputum samples (95%), but also in mucoid sputum samples (78%). So, although they conclude that purulent sputum was associated with bacterial growth, the distinction between purulent and mucoid sputum was not sufficient for the distinction between bacterial growth or not.

There are some differences that possibly could explain some of the contradictory results. One explanation might be that contrary to the abovementioned studies, we looked at total bacterial load on a continuous scale and not classified into bacterial growth or not, or infection or not. Another difference is that in the studies by Stockley, Allegra and Soler, patients had not received antibiotic

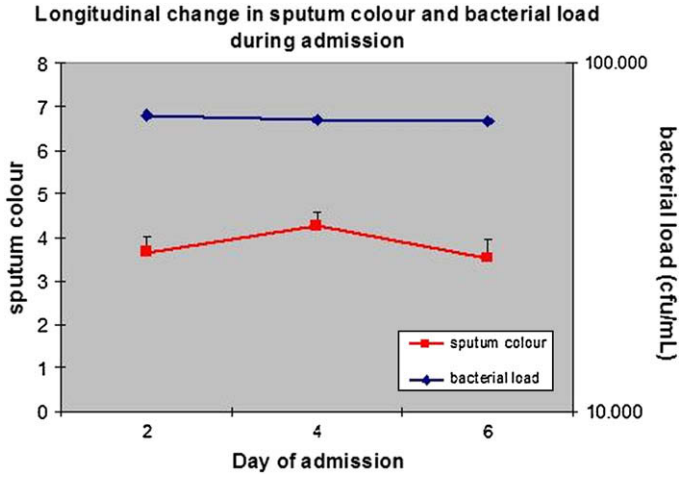

Figure 1 Longitudinal change in mean value (with error bars) of sputum colour and bacterial load during admission.

treatment 4 weeks before admission. In our study 68% of the patients received antibiotics 4 weeks prior to the study and 23% received antibiotics at admission. The decision not to exclude these patients was based on the fact that if we want to use sputum colour as an indication for antibiotic treatment, it should be usable in clinical practice. And as in our study, many patients already receive antibiotics from their GP or chest physician before admission. By performing multivariate analyses, correcting for the use of antibiotics we addressed this item.

Another difference is that Allegra et al. developed their own colour-coding chart, which might have introduced some differences in coding the colour of the sputum samples.

Like in our study, Stockley et al. also looked at changes in sputum colour over time. However, they compared sputum samples during exacerbations and during stable state after 2 months. They showed that sputum colour of patients with mucoid exacerbations remained similar. On the other hand, in patients presenting with purulent sputum, colour changed from a median of 4.0 to a median of 3.0 in stable state. This indicates that many patients do not return to mucoid sputum, which would implicate that these patients would be treated again with antibiotics during their next exacerbation. If one believes in the value of sputum colour determination, this maintenance of purulent sputum should elicit different/longer/higher dose

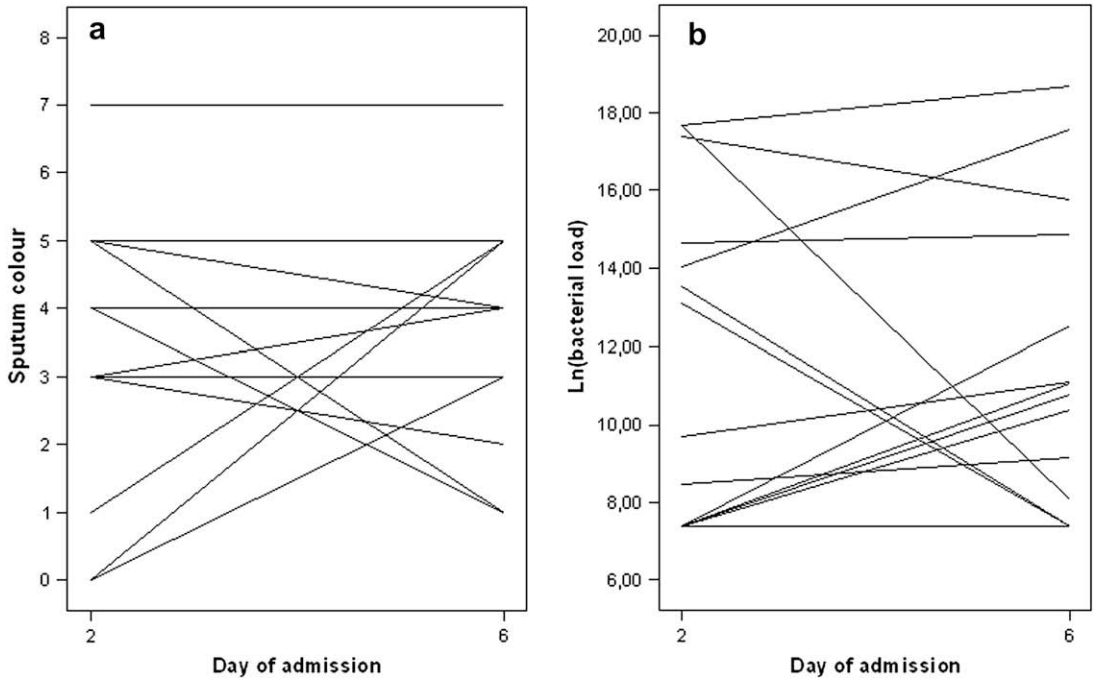

Figure 2 a. Longitudinal change of sputum colour during admission on a patient level. b. Longitudinal change of bacterial load during admission on a patient level.

antibiotics. We, however, observed absolutely no change in both mean sputum colour and mean bacterial load on a group level over the first seven days of admission, although the majority of patients was treated with antibiotics. This might implicate that change in sputum colour takes a long time, and also bacterial load does not seem to be immediately changed by a course of antibiotics. Within-patient changes were, however, often present, but changes were in all directions, and varied from day to day. Furthermore, there appeared to be no consistent relationship between the individual changes in sputum colour and the accompanied change in bacterial load. This makes us reluctant about the usefulness of sputum colour as indicator for bacterial load and therefore for treatment with antibiotics.

The current study showed that there is only a very weak association between bacterial load and sputum colour, which confirms the results of a previous study performed by our study group[1] and that of others. Furthermore when sputum colour was divided into purulent and mucoid sputum, the relationship with bacterial load vanished completely. The distinction between purulent and mucoid sputum at the moment patients present themselves with an exacerbation seems therefore insufficient for distinction between patients who are likely to benefit form antibiotic therapy and those who are not. More studies are needed to determine which easy measurable factors could be used as a predictor for an indication for use of antibiotics; sputum colour is not the one.

Conflict of interest

There is no conflict of interest.

References

1. Van der Valk P, Monninkhof E, van der Palen J, Zielhuis G, van Herwaarden C, Hendrix R. Clinical predictors of bacterial involvement in exacerbations of chronic obstructive pulmonary disease. *Clin Infect Dis* 2004 Oct 1;**39**(7):980–6.
2. Seemungal TA, Donaldson GC, Bhowmik A, Jeffries DJ, Wedzicha JA. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000 May;**161**(5):1608–13.
3. Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998 May;**157**(5 Pt 1):1418–22.
4. Greenberg SB, Allen M, Wilson J, Atmar RL. Respiratory viral infections in adults with and without chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000 Jul;**162**(1):167–73.
5. Greenberg SB. Viral respiratory infections in elderly patients and patients with chronic obstructive pulmonary disease. *Am J Med* 2002 Apr 22;**112**(Suppl. 6A):28S–32S.
6. Seemungal TA, Harper-Owen R, Bhowmik A, Jeffries DJ, Wedzicha JA. Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. *Eur Respir J* 2000 Oct;**16**(4):677–83.
7. Saint S, Bent S, Vittinghoff E, Grady D. Antibiotics in chronic obstructive pulmonary disease exacerbations. A meta-analysis. *JAMA* 1995 Mar 22;**273**(12):957–60.
8. Sethi S, Murphy TF. Bacterial infection in chronic obstructive pulmonary disease in 2000: a state-of-the-art review. *Clin Microbiol Rev* 2001 Apr;**14**(2):336–63.
9. Fagon JY, Chastre J, Trouillet JL, Domart Y, Dombret MC, Bornet M, et al. Characterization of distal bronchial microflora during acute exacerbation of chronic bronchitis. Use of the protected specimen brush technique in 54 mechanically ventilated patients. *Am Rev Respir Dis* 1990 Nov;**142**(5):1004–8.
10. Stockley RA, O'Brien C, Pye A, Hill SL. Relationship of sputum color to nature and outpatient management of acute exacerbations of COPD. *Chest* 2000 Jun;**117**(6):1638–45.
11. Anthonisen NR, Manfreda J, Warren CPW, Hershfield ES, Harding GKM, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann Intern Med* 1987;**106**:196–204.
12. Stockley RA, Bayley D, Hill SL, Hill AT, Crooks S, Campbell EJ. Assessment of airway neutrophils by sputum colour: correlation with airways inflammation. *Thorax* 2001 May;**56**(5):366–72.
13. Allegra L, Blasi F, Diano P, Cosentini R, Tarsia P, Confalonieri M, et al. Sputum color as a marker of acute bacterial exacerbations of chronic obstructive pulmonary disease. *Respir Med* 2005 Jun;**99**(6):742–7.
14. Soler N, Agusti C, Angrill J, Puig De la BJ, Torres A. Bronchoscopic validation of the significance of sputum purulence in severe exacerbations of chronic obstructive pulmonary disease. *Thorax* 2007 Jan;**62**(1):29–35.
15. van der Lende R, Jansen-Koster EJ, Knijpstra S, Meinesz AF, Wever AM, Orie NG. Prevalence of cold in Vlagtwedde and Vlaardingen (computer diagnosis versus doctors' diagnosis). *Ned Tijdschr Geneeskd* 1975 Dec 13;**119**(50):1988–96.
16. Isenberg HD. *Clinical microbiology procedures handbook*; 2004.